REMARKS/ARGUMENTS

Claims 1-15 are active. A minor edit has been made to claim 1 adding a ":". No new matter has been introduced. Favorable consideration of these amendments and allowance of the case are respectfully requested.

Lack of Unity/Restriction/Election

The Applicants previously elected with traverse for examination purposes the species ***rocR*** as a *Bacillus subtilis* gene. The species ***slr*** and ***sigL*** have been rejoined. The claims are directed to microorganisms and not to these genes *per se* so the Applicants construe this requirement as referring to a recombinant microorganism in which *rocR*, *slr*, or *sigL* (or their equivalents) have been deleted or knocked-out. The requirement has been made FINAL. The Applicants point out that the Office has provided no reasoning as to why microorganisms sharing a functional ability to express a heterologous protein or polypeptide and other significant biological features associated with this ability are patentably distinct. Therefore, it is the Applicants' understanding that additional species will be rejoined and examined upon an indication of allowability for a generic claim reading on the elected species. The Applicants also respectfully request that claims which depend from or otherwise include all the limitations of an allowed elected claim, be rejoined upon an indication of allowability for the elected claim, see MPEP 821.04.

Rejection—35 U.S.C. §103(a)

Claims 1-15 were rejected under 35 U.S.C. §103(a) as being unpatentable over Ferrari, et al., WO 03/083125, in view of Gardan, et al., Mol. Microbiol. 24:825 and Hakamada, et al., Biosci. Biotechnol. Biochem. 64:2281.

Ferrari was relied upon for teaching a method for producing a secreted protein in recombinant *Bacillus subtilis* that contains a deletion of the *slr* gene and a gene encoding a heterologous protein. The *slr* gene is one of the species under examination, *rocR* (the elected species) and *sigL* are the other two species under examination. Claim 1 has been amended and no longer requires a *slr* knock out, but does encompass a microorganism with a deletion or knock-out of *rocR* or *sigL*.

Ferrari does not teach deleting or knocking out *rocR* or *sigL*. The Examiner acknowledges this deficiency at the top of the 2nd paragraph on page 3 of the final OA. None of the prior art teaches deleting *rocR* or *sigL* to produce a strain that expresses an increased amount of a heterologous protein.

Nevertheless, the Examiner maintains that deleting *rocR* or *sigL* is suggested by the combination of Gardan and Ferrari.

Gardan teaches two operons involved in arginine catabolism are SigL dependent and require the transcriptional activator RocR.

Ferrari page 2, lines 24-29, describes possible inactivation of numerous genes including *rocA*, *rocD* or *rocF* which form parts of the arginine catabolism operons disclosed by Gardan.

The Examiner argues that knocking out *sigL* or *rocR*, which activate the operons involved with catabolizing arginine (e.g., that permit a cell to break down arginine and use it as a nitrogen source), would have been expected to prevent arginine catabolism by the gene products of *rocA*, *rocD* or *rocF*, and thus enhance heterologous protein expression. The

Examiner deems enhancement of heterologous protein expression to be a "predictable result" (OA, page 3, bottom of 2nd paragraph).

The Examiner's argument fails to make a *prima facie* case for obviousness for several reasons.

First, there is no express teaching, suggestion or motivation in the prior art to knock out *sigL* or *rocR* to produce a *Bacillus* strain that expresses a higher amount of a heterologous protein than a strain not having a *sigL* or *rocR* knock out. Thus, the rejection as based on both Ferrari, in view of Gardan fails the first prong of the test in *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991) that the references themselves or the knowledge in the art must provide some suggestion or motivation to arrive at the invention.

Moreover, the Examiner has not articulated any reason why one of ordinary skill in the art would have *selectively* inactivated *rocR* or *sigL* (as a means of selectively inactivating *rocA*, *rocF* or *rocD* of Ferrari) as opposed to directly inactivating any of the numerous other genes described on page 3, lines 28-29 of Ferrari. Rather, the Examiner engages in hindsight reasoning using the present disclosure as a guide for specifically selecting an indirect knockout of a *rocA*, *rocF* or *rocD* gene regulated by *sigL* or *rocR*. However, one cannot use hindsight reconstruction to pick and choose among isolated disclosure in the prior art to deprecate the claimed invention, see *In re Fine*, 5 USPQ2d 1596 at 1600 (Fed. Cir. 1988);

> It is essential that "the decision maker forget what he or she has been taught at trial about the claimed invention and cast the mind back to the time the invention was made . . . to occupy the mind of one skilled in the art who is presented only with the references, and who is normally guided by the then-accepted wisdom in the art." *Id* . One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention.

Moreover, in the recent Supreme Court decision in *KSR International Co. v. Teleflex Inc.*, 82 USPQ2d 1385 (U.S. 2007) the court noted that "The TSM test captures a helpful insight: A patent composed of several elements is not proved obvious merely by

demonstrating that each element was, independently, known in the prior art." While the court rejected a rigid application of the teaching-suggestion-motivation (TSM) test in *KSR*, it did not relieve the Examiner of the burden of establishing a *prima facie* case based on the scope and content of prior art, ascertainment of the differences between prior art and claims at issue, resolution of the level of ordinary skill in the pertinent art, and any secondary considerations. The Examiner has not met this burden because she has not established that based on the cited prior art and the level of ordinary skill in the art that one would have had a reasonable expectation of obtaining a *Bacillus* strain having an enhanced ability to express a heterologous protein by knocking out *sigL* or *rocR*. For example, assuming *arguendo* that knocking out *sigL* or *rocR* would have inactivated the operons containing the *rocA*, *rocF* and *rocD* genes of Ferrari[1], as assumed by the Examiner, the Examiner has not explained why this would enhance protein synthesis by a *Bacillus* as opposed to either having no effect on protein synthesis or crippling protein synthesis by the cell. Why would one of ordinary skill in the art expect to *increase* protein synthesis by inactivating arginine catabolism pathways? The Examiner has not explained this.

On the other hand, the Applicants have already pointed out reasons why one with ordinary skill in the art would not have expected to increase protein synthesis by knocking out *sigL* or *rocR*—namely that if knocking out these genes knocks out expression of the arginine catabolism genes like *rocCE* (arginine permease), then less arginine would have been expected to be transported into the cell. Less arginine transport would have been expected to result in a lower intracellular level of arginine being available for protein synthesis and thus slower protein synthesis. While the Examiner states at the bottom of page 6 of the OA that *Bacillus* need not use arginine as a nitrogen source, since "*B. subtilis* cells having inactivated *rocR* or *sigL* do grow when other nitrogen sources are used; the necessary

[1] This has not been established by the Examiner.

amino acids are biosynthesized by the cells and protein synthesis is not affected (see Debarbouille, et al.)" (OA, bottom of page 6), this argument confuses the ability of a cell to survive (grow) on a medium containing a particular nitrogen source with the ability of a cell to express higher amounts of a heterologous protein. It does not follow that a cell that can grow on an arginine-free medium (e.g., a cell where the arginine catabolism pathways are knocked out) will produce a higher amount of a heterologous protein than a cell that contains these active pathways.

While it may be perfectly reasonable to expect that a *Bacillus* that does not express arginine permease (or *roc* genes that catabolize arginine) will grow on a medium containing an alternative nitrogen source such as those named on page 9095, col. 1, third full paragraph of Debarbouille, the Examiner has not pointed out any teaching in the prior art that indicates that heterologous protein synthesis would be enhanced in such cells. For example, one of ordinary skill in the art would recognize that cells not able to take in arginine from the medium would have to expend metabolic resources to synthesize arginine they incorporate into a heterologous protein. Cells that can obtain arginine freely from the medium would not be subject to the same metabolic costs and would be expected to more efficiently express heterologous proteins. Consequently, contrary to the unsupported assertion of the Examiner, one of ordinary skill in the art would have expected that knocking out *rocR* or *sigL* would have inhibited the expression of arginine permease and resulted in lower not higher cytoplasmic levels of arginine and lower levels of protein expression.

On the other hand, in contrast to the lack of a reasonable expectation of success for increasing the synthesis of a heterologous protein by knocking out *sigL* or *rocR* in the prior art references, the Applicants have shown that deletion of the *sigL*, *rocR* and *slr* genes produce microorganisms which surprisingly express much higher levels of heterologous protein that the corresponding wild-type microorganisms not having these deletions.

Evidence of unobvious or unexpected advantageous properties can rebut *prima facie* obviousness, MPEP 716.02(a).

The superior productivity of the microorganism according to the invention is shown in Table 4 from page 26 of the specification reproduced below:

Table 4

Name of deleted gene	Gene ID	Gene size (bp)	Size of deleted fragment (bp)	Amount of produced (secreted) alkaline amylase (relative value)
Cultured for 3 days				
slr	**BG11858**	**459**	**394**	**178**
treR	BG11011	717	656	124
yopO	BG13648	213	169	364
yvaN	BG14069	408	379	148
yvbA	BG14078	273	210	171
None (Wild type)	—	—	—	100
Culture for 5 days (Wild type)				
cspB	BG10824	204	171	195
rocR	**BG10723**	**1386**	**1359**	**215**
sigL	**BG10748**	**1311**	**1256**	**204**
glcT	BG12593	858	811	132
yvdE	BG12414	951	916	127
yacP	BG10158	513	513	110
None (Wild type)	—	—	—	100
Cultured for 6days				
yycH	BG11462	1368	1368	120
licR	BG11346	1926	1889	122
None (Wild type)	—	—	—	100

BG10723 having *rocR* knocked out produced 215% more protein than the corresponding wild-type strain and BG10748 having *sigL* knocked out produced 204% more. The prior art provides no expectation of success for the significantly higher levels of heterologous proteins produced by the recombinant microorganisms of the invention.

In the Examples in the present application, nitrogen sources contained in the culture medium used were tryptone and yeast extract, both of which are generally used and well-balanced nitrogen sources. This culture medium is not a specific medium and if some amino

acids such as arginine, isoleucine, or valine are exhausted, the medium does not provided substitutive amino acids.

Although other amino acids in the medium can substitute the exhausted amino acids as alleged by the Examiner[2], this would only be possible during the early phase of culturing when such amino acids are present (i.e., not yet depleted). In the Examples, the protein productivity of host cells was investigated after 5 day culturing at which time the available nitrogen source in the culture medium had been depleted. Thus, it would be clear to one of skill in the art that the amount of the available nitrogen source in a culture medium may affect the productivity to some extent.

Under the culture conditions described above, i.e., the condition in which cells were cultured long enough to deplete all available nitrogen sources in a medium having a normal amino acid content, it would have clearly been expected that the protein productivity of the knocked-out cells which cannot use arginine, isoleucine, or valine would have been lower that that of normal cells which can use these amino acids. Therefore, even taking into account the Examiner's allegation, the higher protein productivity of the claimed microorganism is surprising.

Hakemada was cited with regard to claims 5 and 6 which require regulatory gene sequences from a cellulase gene. However, it does not remedy the deficiencies in the two primary references.

In view of the above, this rejection cannot be sustained in view of the amendments above and the lack of a suggestion or reasonable expectation of success for the superior protein expression provided by the claimed recombinant microorganisms.

[2] See the paragraph on the bottom half of page 6 of the OA.

Conclusion

This application presents allowable subject matter and the Examiner is respectfully requested to pass it to issue. The Examiner is kindly invited to contact the undersigned should a further discussion of the issues or claims be helpful.

Respectfully submitted,

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